The use of conjunctival ultraviolet autofluorescence (CUVAF) as a biomarker of time spent outdoors.

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Abbreviated Title: CUVAF as a biomarker of time spent outdoors

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Purpose: CUVAF has been used in previous Southern Hemisphere myopia research as a marker for time spent outdoors. The validity of CUVAF as an indicator of time spent outdoors is yet to be explored in the Northern Hemisphere. It is unclear if CUVAF represents damage attributed to UV exposure or dry eye. This cross-sectional study investigated the association between CUVAF measures, self-reported time spent outdoors and measures of dry eye.

Methods: Participants were recruited from University staff and students (n=50, 19-64yrs; mean 41). None were using topical ocular medications (with the exception of dry eye treatments). Sun exposure and dry eye questionnaires (Ocular Surface Disease Index (OSDI) and McMonnies) were completed by the participant. Dryness was also assessed using slit lamp biomicroscopy and invasive tear break up time (ITBUT). Images of the temporal and nasal conjunctiva from the right and left eye were captured using a bespoke photography system. The total CUVAF area, average CUVAF pixel intensity per mm^2 and total CUVAF pixel intensity were analysed using MATLAB R2013a (The MathWorks Inc).

Results: Of the 50 participants, 42% were classified as having dry eye. Self-reported sunglass use was negatively associated with all CUVAF measures (Kruskal Wallis total CUVAF area, p=0.04, p_{trend}=0.03, average CUVAF pixel intensity p=0.02, p_{trend}= 0.02, total CUVAF pixel intensity: p=0.04, p_{trend}=0.02). Time spent outdoors was positively associated with all CUVAF measures (Spearman's corr. total CUVAF area: r=0.37, p=0.01, average CUVAF pixel intensity: r=0.36, p=0.01, total CUVAF pixel intensity: r=0.37, p=0.01) and remained significant when sunglass use was controlled for (partial correlation, total CUVAF area: r=0.32, p=0.03, average CUVAF pixel intensity: r=0.39, p=0.01, total CUVAF pixel intensity: r=0.39, p=0.03). Neither CUVAF area nor intensity measures were associated with any dry eye measure (OSDI: all p≥0.41, corneal staining: all p≥0.38, McMonnies: all r≤0.09 all p≥0.52, slit lamp biomicroscopy: all r≤0.20 all p≥0.17, ITBUT: all r≤-0.07 all p≥0.31).

Conclusions: CUVAF area and intensity were not associated with clinical measures of dry eye. Greater CUVAF area and intensity were associated with wearing sunglasses less frequently and spending more time outdoors. If sunglass wear is accounted for, CUVAF may be a useful biomarker of time spent outdoors in future myopia studies.

Conjunctival ultraviolet autofluorescence: A biomarker of time spent outdoors.

Introduction

Due to the increasing prevalence of myopia worldwide, the World Health Organisation (WHO) has recently declared myopia a global health concern. [1] Recent studies have suggested that greater time spent outdoors is protective against myopia. [2-6] Exposure to light of a greater intensity may contribute to the protective effects of time spent outdoors against myopia onset. This has been evidenced in a recent intervention study which increased the intensity of indoor lighting in schools and consequentially decreased the incidence of myopia.[7]

Researchers investigating the impact of environment on myopia development and its progression have utilised a variety of methods to document or estimate the amount of time participants spend outdoors. [4,6,8-10] One group has used conjunctival ultraviolet autofluorescence (CUVAF) as a biomarker for the amount of time spent outdoors. They reported a strong association between CUVAF and myopia in individuals on the Norfolk Island, Australia and mainland Australia.[9,11] Those individuals with highest levels of CUVAF had the lowest prevalence of myopia and the researchers suggested that this relationship indicates that CUVAF is a useful, objective biomarker for outdoor light exposure.[9,10] However, research investigating the validity of CUVAF as an indicator of time outdoors is limited to the Southern Hemisphere.[9,12]

CUVAF photography derives from Wood's lamp, originally used to assess dermatological damage.[13] It is based on the premise that conjunctival components may emit visible fluorescence, seen as CUVAF, upon alteration to their structure by UV exposure.[13,14] Changes to the intracellular content of proteins within the conjunctival matrix may also contribute to the autofluorescence. Such proteins may include cytokines, growth factors and matrix metalloproteinases (MMPs), also suggested to be implicated in pterygium pathogenesis.[15]

The association between pterygium, as a marker of UV exposure, and CUVAF appears to be supported by the majority of research published to date.[16,17] However, in one Australian study a sub group of participants who already had established

pterygia were found not to have any areas of CUVAF.[18] The authors suggested these pterygia were ‘burned out’ and CUVAF may only represent biologically active areas of UV damage. The precise mechanism implicated in CUVAF is unknown and requires further evidence.

Pinguecula is a degenerative conjunctival condition also associated with chronic UV exposure.[19] The association between pingueculae and CUVAF has been explored in Australian children aged three to 15 years.[20] The study reported that CUVAF area was positively associated with pingueculae. Furthermore, CUVAF was also observed in children without pingueculae. The authors suggest that CUVAF is a sensitive indicator of occult conjunctival UV damage prior to the appearance of pingueculae.

CUVAF may be associated with conjunctival damage attributed to ocular surface dryness rather than, or in combination with, UV damage. Pterygium and pingueculae, which are associated with increased areas of CUVAF, have also been associated with dryness.[21,22] Ocular dryness may lead to structural changes within the conjunctiva such as chemosis, thickening, cell metaplasia and loss of goblet cells.[23,24] Research is yet to investigate if structural changes mediated by ocular dryness may contribute to fluorescence measured in CUVAF.

The aim of this study was to explore the association between CUVAF and outdoor light exposure identified through self-report of time spent outdoors, sun exposure habits and methods of sun protection such as wearing sunglasses and wearing a hat. Research to date in the Northern Hemisphere is limited to one study. [25] We investigated these factors in a Northern Hemisphere population and CUVAF intensity as well as CUVAF area has been included in analyses for the first time. The study also investigated whether CUVAF was associated with clinical measures of dry eye commonly used in practice.

Methods

Ethical approval was granted from the Ulster University’s Research Ethics Committee. Participants aged over 18 years were recruited and tested over the months of April and May. Those using topical ocular medications (with the exception of dry eye treatments) were excluded due to potential disruption of the tear film.

Participants using dry eye treatment were asked to refrain from using their treatment on the day of their participation.

CUVAF Photography

A novel, bespoke photography system was developed (Fig 1) using a similar set up to that described by Coroneo and colleagues. [18,20]

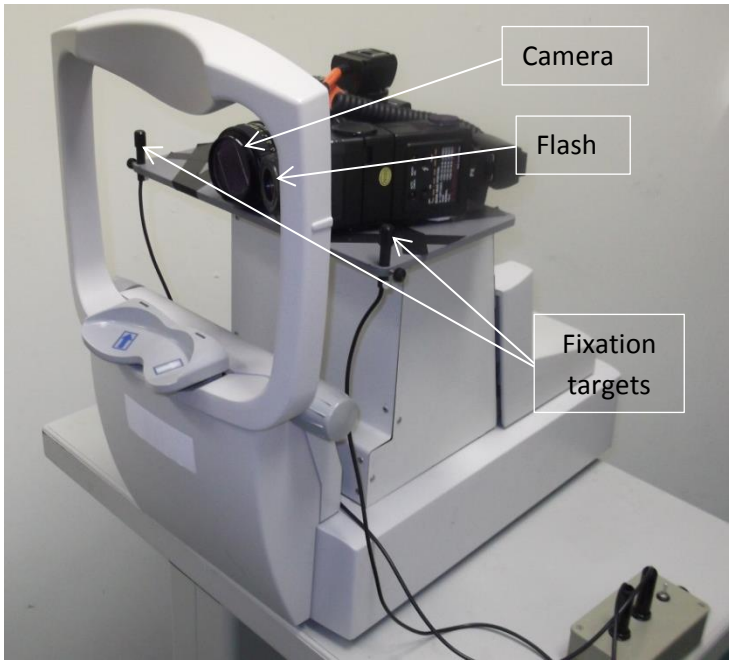

Figure 1. Novel CUVAF photography system used in methodology.

A Sony Nex 6 (Sony, Tokyo, Japan) digital camera and 50mm f/22 lens with a macro extension tube were specially adapted with a UV filter (http://www.edmundoptics.co.uk/optics/optical-filters/longpass-edge-filters/fluorescence-dichroic-filters/86330/) and infrared filter (http://www.edmundoptics.co.uk/optics/optical-filters/shortpass-edge-filters/ir-cut-off-filters/54517/) to ensure that only fluorescence in the visible spectrum was captured. An optimum camera setting of f22 3200 ISO sensitivity was used and images were taken in a dark room to ensure ambient visible light did not interfere with the imaging of fluorescence.

A unidirectional Xenon flash (Centon, FG30D), which ensured minimal reflections, was specially adapted using two UV filters (https://www.thorlabs.de/thorproduct.cfm?partnumber=FGUV11), (http://www.edmundoptics.co.uk/optics/optical-filters/shortpass-edge-filters/high-

performance-od-4-shortpass-filters/84702/) to create an excitation source (transmittance range 275-375nm, peak 330nm). Pilot work identified that there was no variation in flash output between successive flash outputs or across the lifespan of the battery.

The camera and flash system were placed on marked positions on a fixed plate to ensure consistency of imaging. The fixed plate was mounted on a moveable base with a two position chin rest which permitted head rotations to ensure images were unhindered by facial anatomy. LED fixation targets were used to ensure stable fixation.

Images were saved in coloured format at Raw (minimally processed) settings before being converted to a lossless format (TIFF file) for analysis. At least three images of both the temporal and nasal conjunctiva of the right and left eye were captured. The highest quality photograph from each position was chosen for analysis. Images were rejected if the visibility of CUVAF was hindered by lid position or defocus.

CUVAF Image analysis

A commercial software package (MATLAB, The MathWorks Inc., Natick, MA, 2013) was used to determine the area as well as the intensity of the fluorescence captured. The repeatability of this technique has been determined previously. [26] Images were analysed by the same observer. Firstly, an area encompassing the fluorescence was subjectively outlined (Fig 2)

Figure 2. Sample image used in analysis. An area encompassing the CUVAF has been subjectively outlined prior to MATLAB analysis.

An algorithm was created with MATLAB to determine a pixel threshold that provided an automated means of differentiating fluorescence from non-fluorescence within the outlined area. The area of fluorescence in pixels was then determined by MATLAB. Finally, the CUVAF pixel area was converted to mm^2 using an algorithm that accounted for camera magnification.

Total CUVAF area (mm^2) for an individual was calculated by summing the temporal and nasal areas of the right and left eye.

To explore CUVAF intensity, two matrices were calculated from the images; the average CUVAF pixel intensity per mm^2 and the total CUVAF pixel intensity across the fluorescing area. As the average CUVAF pixel intensity value determines the pixel intensity per unit area of CUVAF, it has the ability to discriminate between those with small bright areas of CUVAF and those with large dim areas of CUVAF. The values from both nasal and temporal images were used to determine an individual's average CUVAF pixel intensity per mm^2 and the total CUVAF pixel intensity.

Self-reported Sun Exposure Questionnaire

Participants completed a validated questionnaire [27,28] relating to the frequency of time spent outdoors, sun exposure habits, whether they consistently wore sunglasses and/or a hat. Questions applied to the Spring and Summer months (April to September) of the past year.

A total score for frequency of time spent outdoors was calculated. Participants selected how often they were outdoors for at least half an hour for each section of the day ('before 10am, 10am-3pm and after 3pm') and how often during the week this occurred ('less than once a week, 1-2x per week, >2 x per week, everyday'). A higher score corresponded with greater time spent outdoors.

Sun exposure habits were categorised as 'avoids the sun, sometimes stays in the sun, often stays in the sun'. Wearing sunglasses and wearing a hat were categorised as 'never, rarely, sometimes, usually, always' for each variable.

Subjective measurement of dry eye

The previously validated Ocular Surface Disease Index (OSDI) [29] and McMonnies [30] questionnaires were used to subjectively explore dry eye. The questionnaires were completed by the participant and a score calculated for each. The observer was masked to questionnaire results during examiner assessment of dry eye.

The OSDI consists of 12 questions, with a possible score of 0-100, pertaining to dry eye symptoms in different environments and when completing various tasks. The score calculated was used to classify the presence or absence of dry eye as a binary outcome using the OSDI classification system. The test-retest reliability of OSDI has been previously determined to be good to excellent by intra-class correlation (ICC). [29]

The McMonnies questionnaire consists of 14 questions, with a possible score of 0-45. It pertains to dry eye symptoms, medications and health conditions. The test-retest reliability of McMonnies questionnaire has been previously determined to be moderate by ICC. [30]

Examiner measurement of dry eye

Slit lamp biomicroscopy was used to assess the lids and conjunctiva for signs of meibomian gland dysfunction, inflammation and swelling. Findings were graded according to Foulk and Bron. [31] Details of the grading scale have been published by Moore *et al.* [32.]

Invasive tear break up time (ITBUT) was measured with 1mg fluorescein sodium paper strips using standard methodology. [32] Strips were dampened using saline, shaken and pressed to the inferior conjunctiva and then removed. The participant was asked to blink and the time taken for a break to appear in the tear film, under cobalt blue light, was recorded in seconds. This measurement was repeated three times per eye and the average calculated. The presence or absence of corneal staining was also recorded.

Statistical methods: Sample size

The 95% limits of agreement derived from the intraobserver repeatability of total CUVAF area measures[26] were used to inform sample size calculations (power of 90%, significance 5%). The results indicated that 17 participants with dry eye and 17

participants without dry eye would be sufficient to determine differences in total CUVAF area between those with and those without dry eye.

Statistical methods: Analyses

Total CUVAF area, average CUVAF pixel intensity and total CUVAF pixel intensity measures were analysed as continuous variables. As CUVAF area and pixel intensity measures were not normally distributed (Skewness and Kurtosis Test: total area $p=0.0022$, average pixel intensity $p=0.047$, total pixel intensity $p<0.001$), analyses were performed using non-parametric tests.

CUVAF (area and pixel intensity measures) and binary variables (OSDI presence/absence of dry eye, presence/absence of corneal staining and male/female gender) were analysed using Wilcoxon rank-sum test. Spearman's correlation was used to assess the relationship between continuous variables (McMonnies, biomicroscopy grading, ITBUT, time outdoors score, age) and CUVAF (area and pixel intensity measures). Correlation coefficients (r) are reported for all correlation analyses. The Kruskal Wallis test was used to assess differences within categorical variables (wearing sunglasses, wearing a hat, sun exposure habits) and CUVAF (area and pixel intensity measures) and the corresponding degrees of freedom (d.f.) reported. Trends across categories were assessed using Cuzik's non-parametric test for trend (p_{trend}). The Wilcoxon signed rank test was used to assess the difference in area and pixel intensity measures between temporal CUVAF and nasal CUVAF.

Statistical tests were performed using a statistical significance of 5% ($p<0.05$). All statistical tests were performed using Stata 13.1 (StataCorp Texas, USA).

Results

Participant characteristics

Participants were recruited from staff and students at Ulster University (n=50), of which 72% were female. All participants were Caucasian and residents of Northern Ireland. The mean age of participants was 41years (19-64yrs, SD 13yrs).

Four participants had no evidence of CUVAF. Median total CUVAF area was 4.9mm^2 (Inter-quartile range (IQR) 2.2-9.4mm^2). Median total CUVAF area temporally was

2.1mm^2 (IQR 0-4.5mm^2) and median total CUVAF area nasally was 2.9mm^2 (IQR 1.5-4.9mm^2).

Median average CUVAF pixel intensity was 312x10^3/mm^2 (IQR 195 x10^3/mm^2 - 380 x10^3/mm^2). Median average CUVAF pixel intensity temporally was 161 x10^3/mm^2 (IQR 0- 185 x10^3/mm^2) and median average CUVAF pixel intensity nasally was 186 x10^3/mm^2 (IQR 169 x10^3-199x10^3/mm^2).

Median total CUVAF pixel intensity was 421x10^3(IQR 207 x10^3-931 x10^3). Median total CUVAF pixel intensity temporally was 168x10^3 (IQR 0-410x10^3) and median total CUVAF pixel intensity nasally was 278x10^3 (IQR 138x10^3-497x10^3).

OSDI questionnaire responses indicated that 42% of participants had dry eye and 48% of participants were found to have corneal staining. Additionally, 43% of the participants classified as having dry eye by the OSDI questionnaire also exhibited corneal staining.

The score calculated from McMonnies was not used to classify the presence of dry eye but was analysed as a continuous variable describing dryness. This method was chosen as McMonnies classification resulted in 78% of participants being classified as having dry eye based on the McMonnies protocol.

CUVAF Characteristics

The total CUVAF area and total CUVAF pixel intensity did not differ temporally and nasally (p=0.10, p=0.07) respectively). However, average CUVAF pixel intensity was greater nasally than temporally (Fig 3).

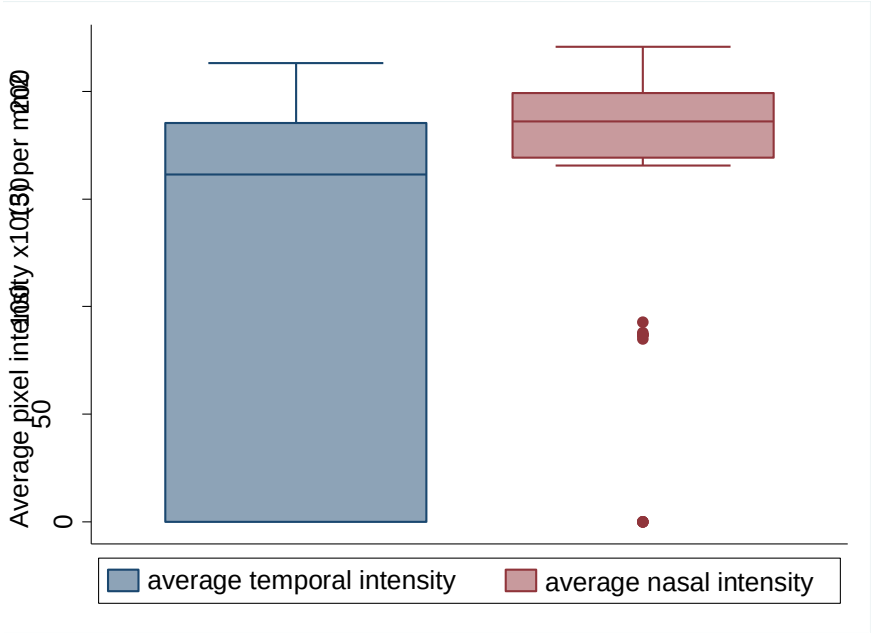

Figure 3 Box plot illustrating distribution of average CUVAF pixel intensity temporally and nasally. There was significantly greater CUVAF pixel intensity nasally compared with temporally (p<0.001).

The difference between nasal and temporal average CUVAF pixel intensity remained significant in those who reported sunglasses use (all p≤0.04). However, there was no difference between average CUVAF pixel intensity measures in the two locations in those who did not report sunglasses use (p=0.26).

Age (yrs) was not associated with total CUVAF area (r=0.05, p=0.74). However, age was positively associated with average CUVAF pixel intensity (Fig 4). Although total CUVAF pixel intensity increased with increasing age, this association was not significant (r=0.10, p=0.49).

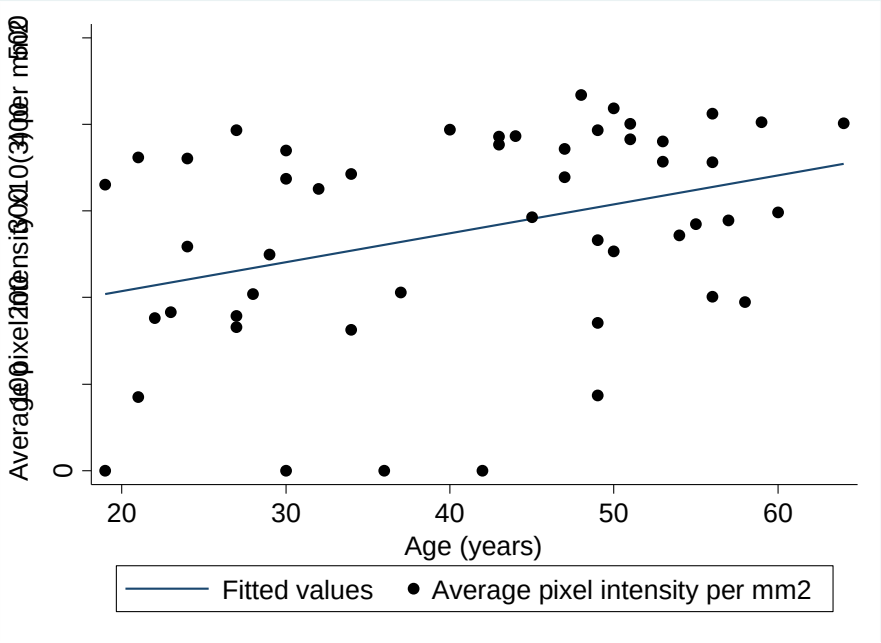

Figure 4 Scatter graph illustrating increasing average CUVAF pixel intensity with increasing age. (r=0.37, p=0.01, R^2 = 0.13; Spearman's correlation).

Gender was not associated with total CUVAF area (p=0.16), average CUVAF pixel intensity (p=0.53) or total CUVAF pixel intensity (p=0.15).

CUVAF and Ocular Dryness

Table 1 provides a summary of data on total CUVAF area, average CUVAF pixel intensity and measures of ocular dryness (OSDI, McMonnies, grading of slit lamp findings, presence of corneal staining and ITBUT), all p≥0.17.

Table 1: Association between CUVAF (total area and average pixel intensity) and ocular dryness n/50= number of responses per category out of a total of 50 for each variable

			Total CUVAF Area		**Average CUVAF pixel intensity**	
	n/50	**%**		**p**		**p**
Ocular Surface Disease Index (OSDI)†						
No dry eye	29	58		0.89		0.41
Dry eye	21	42				

Corneal staining† Not present Present	26 24	58 42		0.56		0.48
			Total CUVAF Area		**Average CUVAF pixel intensity**	
	n/50	**%**	**r**	**p**	**r**	**p**
McMonnies Questionnaire‡	50	100	-0.09	0.53	0.09	0.52
Biomicroscopy grading‡	50	100	0.14	0.34	0.20	0.17
ITBUT‡	50	100	-0.07	0.64	-0.15	0.31

† Wilcoxon Rank-Sum, OSDI: The score calculated was used to classify the presence or absence of dry eye as a binary outcome using the OSDI classification system

‡Spearman's correlation

Results also indicated that total CUVAF pixel intensity was not associated dry eye measures (all p≥0.38).

CUVAF and Sun Exposure Questionnaire

Table 2 provides a summary of data on total CUVAF area, average CUVAF pixel intensity and their association with self-reported data on sun exposure and time spent outdoors. Due to low numbers in the 'never' and 'rarely' sunglass categories and in the 'usually' and 'always' hat categories, responses from these categories were pooled.

Table 2: Association between CUVAF (total area and average pixel intensity) and self-reported measures of sun exposure
n/50= number of responses per category out of a total of 50 for each variable

			Total CUVAF Area (mm^2)					Average CUVAF Intensity ($x10^3$)/mm^2				
	n/50	**%**	**Median (mm^2)**	**d.f.**	**r**	**p**	**p_{trend}**	**Median ($x10^3$)/mm^2**	**d.f.**	**r**	**p**	**p_{trend}**
Frequency sunglasses are worn§												
Rarely/never	8	16	10.5					394				
Sometimes	9	18	4.8	3		0.04	0.03	285	3		0.02	0.02
Usually	17	34	4.3					267				
Always	16	32	3.2					281				
Frequency a hat is worn§												
Never	11	22	5.0					339				
Rarely	15	30	4.3	3		0.77	0.35	183	3		0.34	0.65
Sometimes	17	34	4.5					326				
Usually/always	7	14	9.3					361				
Sun exposure habits§												
Avoids sun	12	24	3.7					261				
Sometimes stays in sun	28	56	5.8	2		0.68	0.50	296	2		0.60	0.32
Often stays in sun	10	20	4.4					357				
Time spent outdoors score‡	50	100			0.37	0.01				0.36	0.01	

§ Kruskal Wallis

‡Spearman's correlation

¶ Wilcoxon Signed Rank

† Wilcoxon Rank-Sum

The frequency with which sunglasses were worn was negatively associated with total CUVAF area (Fig 5), average CUVAF pixel intensity (Fig 6) and total CUVAF pixel intensity (d.f).=3, p=0.04, p_{trend}=0.02).

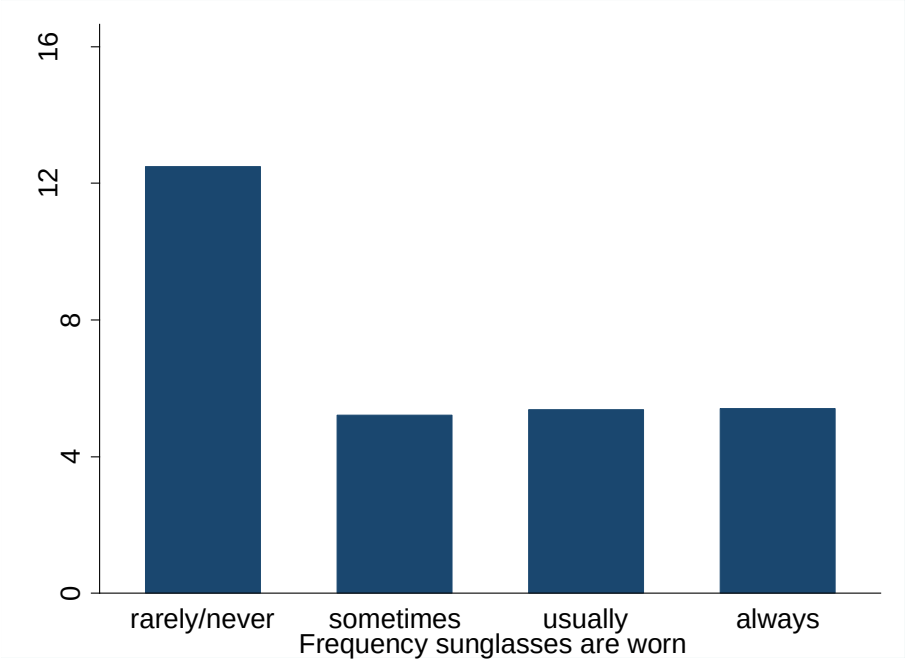

Figure 5 Bar chart illustrating greater total CUVAF area when sunglasses were worn less frequently (d.f.=3, p=0.04, p_{trend}=0.03, Kruskal-Wallis).

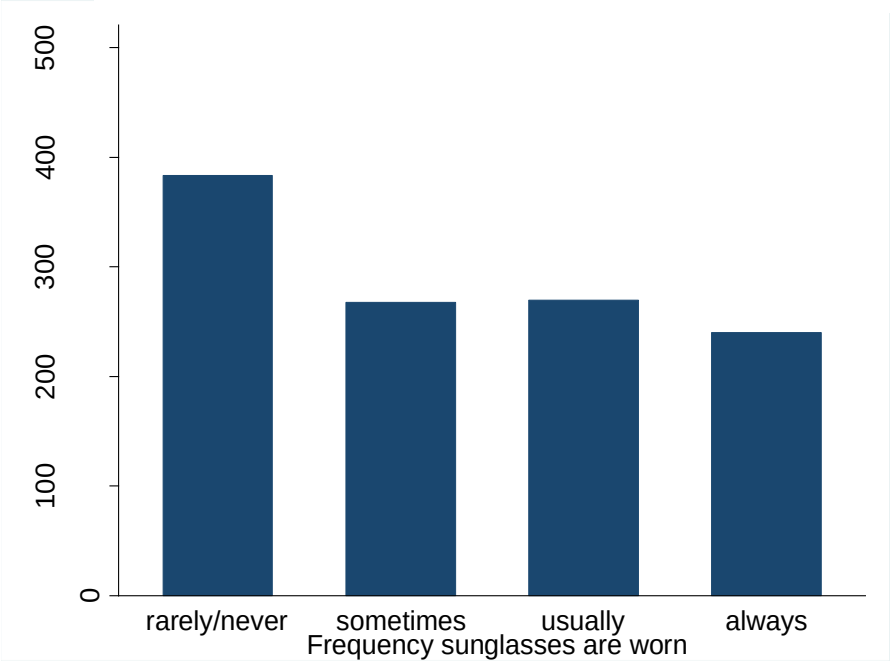

Figure 6. Bar chart illustrating greater average CUVAF pixel intensity when sunglasses were worn less frequently (d.f.=3, p=0.02, p_{trend}=0.02, Kruskal-Wallis).

Additional analyses indicated that only temporal measures of CUVAF were negatively associated with wearing sunglasses (total temporal CUVAF area d.f=3, p=0.01, p_{trend} =0.01, average CUVAF pixel intensity d.f=3, p=0.03, p_{trend} =0.07, total CUVAF pixel intensity d.f=3, p=0.01, p_{trend} =0.01). Nasal measures of CUVAF were not significantly influenced by sunglasses use (all p≥0.11).

However, wearing a hat was not associated with total CUVAF area (d.f=3, p=0.77, p_{trend} = 0.35), average CUVAF pixel intensity (d.f=3, p=0.34, p_{trend} =0.65) or total CUVAF pixel intensity (d.f=3, p=0.74 p_{trend}=0.04).

Sun exposure habits were not associated with total CUVAF area (d.f.=2, p=0.68,p_{trend} =0.50), average CUVAF pixel intensity (d.f.=2, p=0.60, p_{trend} =0.32) or total CUVAF pixel intensity (d.f.=2, p=0.70, p_{trend} =0.46). However, the time spent outdoors score was positively associated with total CUVAF area, average CUVAF pixel intensity and total CUVAF pixel intensity (Fig 7). These relationships remained significant when age and sunglass use were controlled for (partial correlation, total CUVAF area: r=0.32, p=0.03, average CUVAF pixel intensity: r=0.39, p=0.01, total CUVAF pixel intensity: r=0.39, p=0.03).

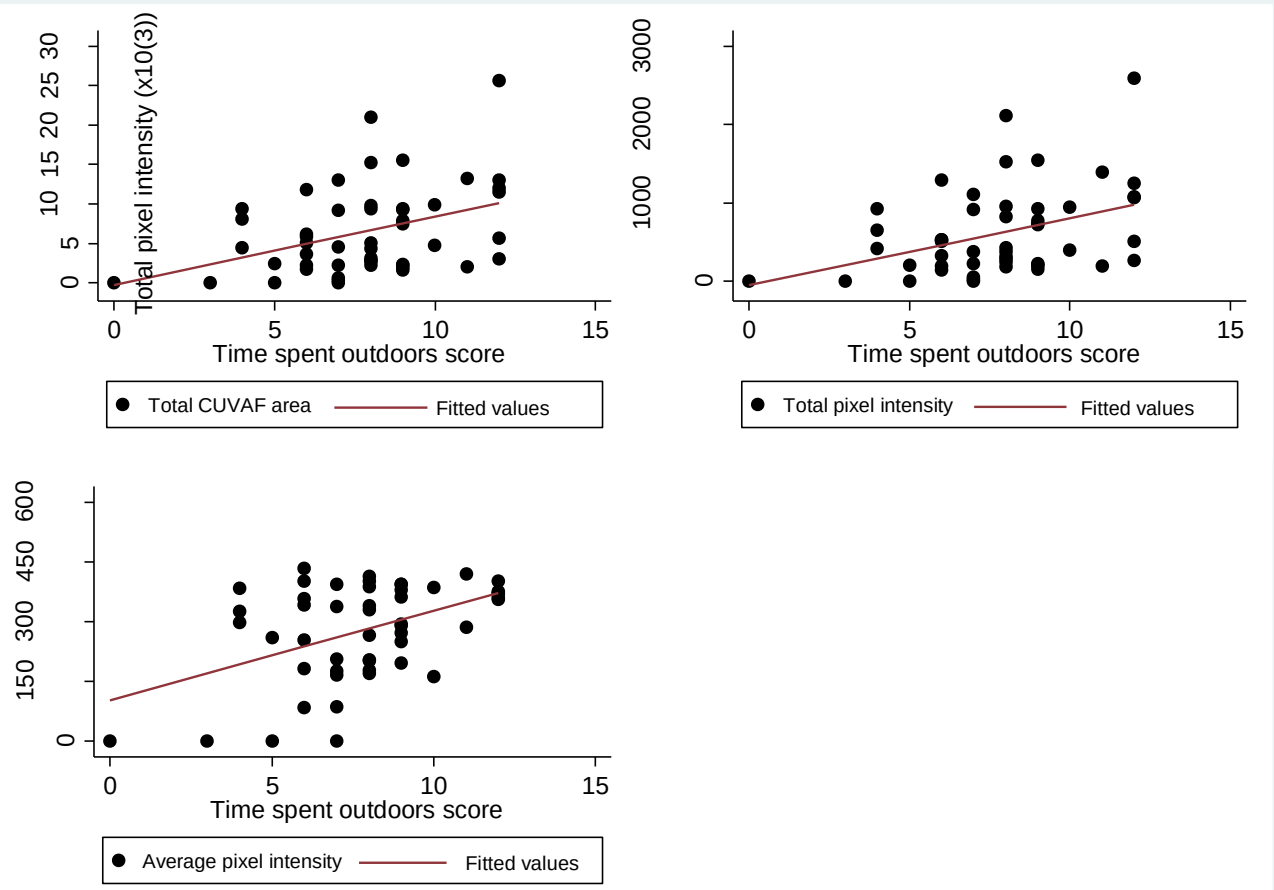

Figure 7. Scatter graphs illustrating increasing total CUVAF area, average CUVAF pixel intensity and total pixel intensity with increasing time outdoors score (r=0.37, p=0.01, R^2 = 0.16); (r=0.37, p=0.01, R^2 = 0.16); (r=0.37, p=0.01 R^2 = 0.15, respectively) Spearman's correlation).

Discussion

CUVAF and sun exposure

The data presented demonstrate that in a Northern Hemisphere country, where there is typically less intense exposure to UV, CUVAF area and intensity are positively associated with self-reported time spent outdoors. This is in agreement with studies in the sunnier climates of Norfolk Island and Australia, [9,12,16,17] where locations such as Sydney experience an average of six to seven hours of daily sunshine all year. [33,34] This is much greater in comparison to Northern Ireland which experiences an average of 1.3 to 2 hours of daily sunshine in the winter months and five to six hours of daily sunshine in the summer months.[35]

To date, the association between CUVAF and sun protection habits is unclear. Decreased areas of CUVAF have not, as might be predicted, been associated with the wearing of sunglasses or sunhats in the Northern or Southern Hemispheres. [10,11,17,25] Conversely, one Australian study reported wearing a hat to be positively associated with CUVAF. [17] The current study reported that CUVAF area and intensity measures were negatively associated with participant report of sunglass wear. This suggests that wearing sunglasses may be protective against CUVAF. Information on the coverage and protection provided by the sunglasses worn will allow for further exploration of the equivocal association between CUVAF and sunglasses in future studies. Conversely, a significant association between CUVAF and wearing a hat was not reported in agreement with Sherwin *et al* [11]. As the majority of participants reported wearing a hat only rarely or sometimes, further information is required to explore the extent of CUVAF in those who wear a hat more regularly. However, the ability of UV light to penetrate fabric may explain the presence of CUVAF even in those wearing a hat.[36]

The association between self-reported sunbathing habits (using ‘sun-worshipper’, ‘average sun exposure’ and ‘sun avoider’ classifications) and CUVAF has previously been explored in participants residing in the Northern and Southern Hemispheres. [25] The results did not indicate a significant association between sunbathing habits and CUVAF area. The current study also reported that CUVAF measures were not associated with sun seeking behaviour. The questions pertaining to sun exposure habits categorised the participants into those who avoided the sun, those who

sometimes stayed in the sun or those who often stayed in the sun. These responses do not provide information on the length of time the individual was exposed to outside light and further research quantifying UV exposure objectively using dosimetry[37] and CUVAF would be beneficial. Exposure to sunlight through habitual time spent outdoors may be sufficient to result in CUVAF regardless of sun seeking habits. It may also be suggested that the amount CUVAF is influenced by other factors in addition to sun seeking habits.

A genetic susceptibility may also promote CUVAF in addition to UV damage acquired through sun exposure. Yazar *et al*. [38] explored the hereditability of CUVAF in twins participating in the Twins Eye Study in Tasmania (n=146, mean 12 yrs, range 5-51yrs), the Brisbane Adolescent Twin Study (n= 444, mean 19yrs, 13-28yrs) and singletons recruited in the Raine study (n=661, mean 20 yrs,18-22yrs).The results indicated that although UV exposure contributed most significantly to CUVAF area; genetics were also associated with CUVAF area after accounting for age and gender. The authors postulated that whilst a genetic predisposition to CUVAF may exist within the population, exposure to UV light has the greatest influence on the pathogenesis of CUVAF.

The use of refractive correction including UV blocking contact lenses and spectacle lenses including photochromatics may also be protective against CUVAF. Although these variables were not explored in the current study, McKnight *et al* [9] reported that corrective lenses did not protect against CUVAF nor were they associated with greater areas of CUVAF. Wolffsohn *et al* [25] also reported that temporal CUVAF area was not significantly related to spectacle and contact lens wear in a study spanning both Northern and Southern hemisphere countries. However, nasal CUVAF area was significantly greater in corrected participants. The latter finding could be, at least partially, attributed to the Coroneo effect discussed below.

CUVAF characteristics

Previous authors [17,25] have proposed that CUVAF is greater nasally than temporally due to the peripheral light focussing effect also termed the Coroneo effect.[39] This occurs when scattered light, that is incident on the periphery of the temporal limbus, is intensified as it is refracted across the anterior chamber. The intensity of the

refracted light reaching the nasal limbus is then 20 times greater than the scattered light incident on the temporal limbus.[40]

The average CUVAF intensity measure was found to be greater nasally than temporally in the current study but CUVAF area and total CUVAF intensity did not differ between these locations. Although previous studies have documented CUVAF area to be greater nasally than temporally[17,25], data on CUVAF intensity are not available for comparison purposes. It may be suggested that average CUVAF intensity may be a more accurate measurement of UV exposure as it pertains to the intensity of the fluorescence per mm^2.

Temporal measures of CUVAF were negatively associated with wearing sunglasses whereas nasal measures of CUVAF were not. Additionally, nasal measures of average CUVAF intensity were not significantly different to temporal measures of average CUVAF intensity in those who did not report sunglasses use. This indicates that wearing sunglasses is not protective against nasal CUVAF possibly due to the Coroneo effect whereby peripheral light rays may still reach the anterior chamber and promote nasal UV damage. Further research is required to explore the use of wraparound sunglasses in protecting against the Coroneo effect.

We also reported that only average CUVAF intensity was positively associated with age, indicating that average CUVAF intensity may reflect accumulative UV damage. Research to date that has investigated CUVAF in relation to age is conflicting and has not included CUVAF intensity in analyses. A positive association between CUVAF area and age was reported in Australian children aged 3 to 15 years. [20] whereas a negative association between CUVAF area and age was reported in adults from the Norfolk Island, Australia aged 16 to 85 years.[41] Sherwin *et al* [41] proposed that CUVAF may be indicative of short-term UV damage, similar to a skin tan. Previous work has reported that UV induced structural changes to skin tissue may be at least partially reversible if excessive UV exposure is avoided whereas repeated exposure to UV light promotes accumulative changes.[14] Further work is required to determine if CUVAF represents long term or short term UV damage.

Research on the Norfolk Island found that CUVAF was greater in males than females.[12,41] The authors suggested that males may be more likely to have an outdoor occupation and to enjoy outdoor recreational activities.[17,41] Moreover, in a

similar cohort of males, a higher prevalence of Ophthalmoheliosis such as pterygium has been documented.[16] However, no gender differences were found in the current study or in previous work in Australia, across Europe and Asia relating to CUVAF measures.[9,25] This may be explained by a similar lifestyle had by both females and males in the selected cohorts.

CUVAF and dry eye

CUVAF area and intensity measures were not associated with dry eye measures. This suggests that dry eye is not a confounder in the use of CUVAF as a biomarker for outdoor light exposure. A high prevalence of dry eye was found, as classified by OSDI and the presence of corneal staining. This may be explained by the characteristics of the participants who were computer users, mainly female and over the age of 40. Such variables have been implicated in dry eye syndrome.[42,43]

Limitations

The current study possessed strengths including the use of a previously validated sun questionnaire in a wide age range of participants. However medications that promote an individual's sensitivity to skin damage from UV radiation, such as tetracyclines, were not accounted for. [44]

Although a variety of dry eye measures were explored in the current study; the measurement of tear osmolarity using a specialised device that collects a small sample of tears may have provided a more objective means of quantifying dry eye. However, the dry eye measures used in this study are inexpensive, widely used and accepted in clinical practice.

Traditional analyses of the OSDI and McMonnies questionnaire investigates the total raw score from the summation of the responses obtained from the rating scale employed in the questionnaire. While this form of analyses has been criticised, [45-47] many researchers and research studies continue to utilise these traditional methods. [48,49]

Conclusion

To the authors' knowledge, this is the first study to have investigated the association between CUVAF and measures of dry eye and to have included CUVAF intensity

measures as well as area in analyses. The results indicate that CUVAF may be a useful indicator of outdoor light exposure. Further work is required to determine the longevity of CUVAF and to explore its use in myopia studies.

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